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HIGH AFFINITY IBOGAINE BINDING TO A MU OPIOID AGONIST SITE

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Abstract. The naturally occurring indole alkaloid ibogaine is of interest because of its reported ability to block drug seeking behavior for extended periods. The compound also potentiates morphine-induced analgesia in mice and reduces certain naltrexone-precipitated withdrawal signs in morphine-dependent rats. Although these results might suggest ibogaine interaction with opioid receptors, previous receptor binding studies (Brain Res. 571:242-247, 1980) found that ibogaine had a K_i value of only 2 μ M for the kappa opioid receptor and was virtually inactive in blocking mu and delta receptor binding ($K_i > 100 \mu$ M). The present investigation of ibogaine interaction with the mu opioid receptor from mouse forebrain labeled with [3 H]-naloxone, however, yielded significantly more potent mu opioid K_i values. LIGAND analysis indicated that the data were best fit by a two site binding model, with K_i values of about 130 nM and 4 μ M, reflecting ibogaine recognition of different agonist affinity states of the receptor. Inclusion of 100 mM NaCl in the assay to induce the agonist low affinity state of the receptor, reduced ibogaine's inhibition of [3 H]-naloxone binding. These results suggest that ibogaine is an agonist at the mu opioid receptor with a K_i value of about 130 nM, potentially explaining ibogaine's antinociceptive effects as well as its reported reduction of opioid withdrawal symptoms and attenuation of drug seeking behavior.

Key words: drug dependence, opioid withdrawal, naloxone, sodium shift

Introduction

Because of clinical interest deriving from ibogaine's reported ability to block drug seeking behavior for extended periods (1,2,3), the alkaloid's mechanism of action is the subject of wide study. Possible interaction with dopaminergic, serotonergic, sigma and opioid systems has been postulated (4,5,6,7,8). While not analgesic itself, ibogaine potentiates morphine-induced analgesia in mice (9) and reduces certain naltrexone-precipitated withdrawal signs in morphine-dependent rats (10,11), suggesting the compound's interaction with opioid receptors.

The in vitro binding of ligands to the mu opioid receptor is affected by the conditions under which the receptor membranes are prepared and the ionic composition of the assay buffer (12). Monovalent cations, especially Na^+ , reduce binding of opioid agonist (but not antagonist) ligands (13,14), apparently by inducing a conformational change in

the receptor (15) that results in a decrease in agonist but not in antagonist affinity for the receptor ("sodium shift").

The present study uses ibogaine inhibition of the binding of the opioid antagonist naloxone as a tool to probe ibogaine's interaction with the opioid receptor. The results reveal not only that ibogaine is a more potent inhibitor of opioid binding than previously thought, but also that the alkaloid appears to be an agonist at opioid receptors.

Methods

Male, Crl:CD-1® mice (VAF, Charles River, Kingston, NY) were sacrificed by cervical dislocation, and their brains removed and placed immediately in ice cold Tris HCl buffer (50 mM, pH 7.4, 23°C). The forebrains were separated from the remainder of the brain by a coronal transection, beginning dorsally at the colliculi and passing ventrally through the midbrain-pontine junction. The forebrains were homogenized in Tris buffer (1 g of forebrain tissue per 100 mL) using a Polytron PT 3000 at 10,000 rpm and centrifuged at 39,000 x g for 10 min. The pellet was resuspended in the same volume of Tris buffer, and 0.5 mL was used per assay tube. [³H]-naloxone (Dupont-New England Nuclear) was used as the radiolabeled ligand. In the ibogaine inhibition experiments, the concentration of [³H]-naloxone was at about its K_d value, 0.4 nM. Saturation curves of [³H]-naloxone binding and ibogaine inhibition curves were performed with and without 100 mM NaCl added to the Tris buffer. Following incubation for 2 hr at 25°C, the assay tube contents were filtered through Whatman GF/B filter sheets (presoaked with 0.5% polyethyleneimine) on a Brandel cell harvester. The tubes and filters were rinsed three times with 4 mL of 10 mM HEPES (pH 7.4), and the radioactivity associated with the filter circles determined using Formula 989 scintillation fluid (New England Nuclear, Boston, MA) in a scintillation counter.

The data were analyzed using LIGAND, a nonlinear curve fitting program designed specifically for the analysis of ligand binding data (16). Nonspecific binding was computed by LIGAND as a fitted parameter. One and, where appropriate, two site models were fit to the data. The goodness of fit of the two models was evaluated with an F-test; the resulting p values are given for those experiments for which a two site fit provides a statistically better fit to the data than a one site fit. LIGAND was also used to evaluate whether the K_d or B_{max} value obtained without added NaCl was significantly different from that obtained in the presence of 100 mM NaCl. Differences between the mean values of two groups were evaluated with a t-test.

Results

The binding of [³H]-naloxone to opioid receptors from mouse forebrain was investigated over the concentration range of 0.04 to 4.0 nM, with triplicates of the ten ligand concentrations assayed. Parallel experiments were performed with and without the addition of 100 mM NaCl to the assay buffer. As can be seen in Table I, K_d values of about 0.5 nM were obtained for [³H]-naloxone binding to mouse brain opioid receptors under both assay conditions. As would be expected for an antagonist, these K_d values were not significantly different from each other. The B_{max} value obtained in the presence of NaCl, however, was almost twice that obtained in Tris buffer without NaCl; these values were significantly different from each other (0.001 < p < 0.01).

TABLE I
Calculated Parameters for [³H]-Naloxone Binding
Under Two Assay Conditions.

	Tris	Tris-NaCl
K_d (M)	4.43E-10	6.16E-10
B_{max} (M)	1.68E-11*	3.33E-11*

* B_{max} values significantly different from each other, 0.001<p<0.01

TABLE II
K_i Values (nM) for Ibogaine Inhibition of [³H]-Naloxone Binding

Parameter	Tris	Tris-NaCl
One site: K_i	2770±467 a,b	4460±339 b
Two sites: K_{i1}	131± 31	
K_{i2}	3760±790	

a Mean±SD (n=3)

b K_i values significantly different from each other, p=0.015

Studies of the inhibition of [³H]-naloxone binding by the alkaloid ibogaine were also performed in Tris buffer and in Tris containing 100 mM NaCl. While the K_i values obtained with single site fits to the data under both assay conditions were in the micromolar range (Table II), the value obtained in the presence of NaCl was almost twice that obtained in Tris buffer alone, suggesting that ibogaine is an agonist at the opioid receptor. These K_i values were significantly different from each other (p=0.015). Furthermore, ibogaine inhibition data obtained in Tris buffer alone were better fit by a two site model (Fig.1) having K_i values of about 130 nM and 4 μM (Table II). In contrast, ibogaine inhibition of [³H]-naloxone binding in Tris-NaCl did not fit a two site model.

Similar results were obtained with studies of morphine inhibition of [³H]-naloxone binding. In single site fits of the data, poorer K_i values were obtained in the presence of NaCl (2.81 nM) than in Tris buffer alone (1.53 nM). Also, morphine inhibition of [³H]-naloxone binding in Tris (but not in Tris-NaCl) was better fit by a two site binding model (K_i values of 0.65 and 42.4 nM; p=0.009 one vs two site fit).

Discussion

The differential effect of Na⁺ on the affinity of opioid agonists and antagonists is a well established tool to determine the functional properties of a ligand, with the ratio of the K_i values with and without Na⁺ ("sodium shift") differentiating agonists from antagonists. Ratios near one typify antagonists, while ratios greater than one are found for agonists

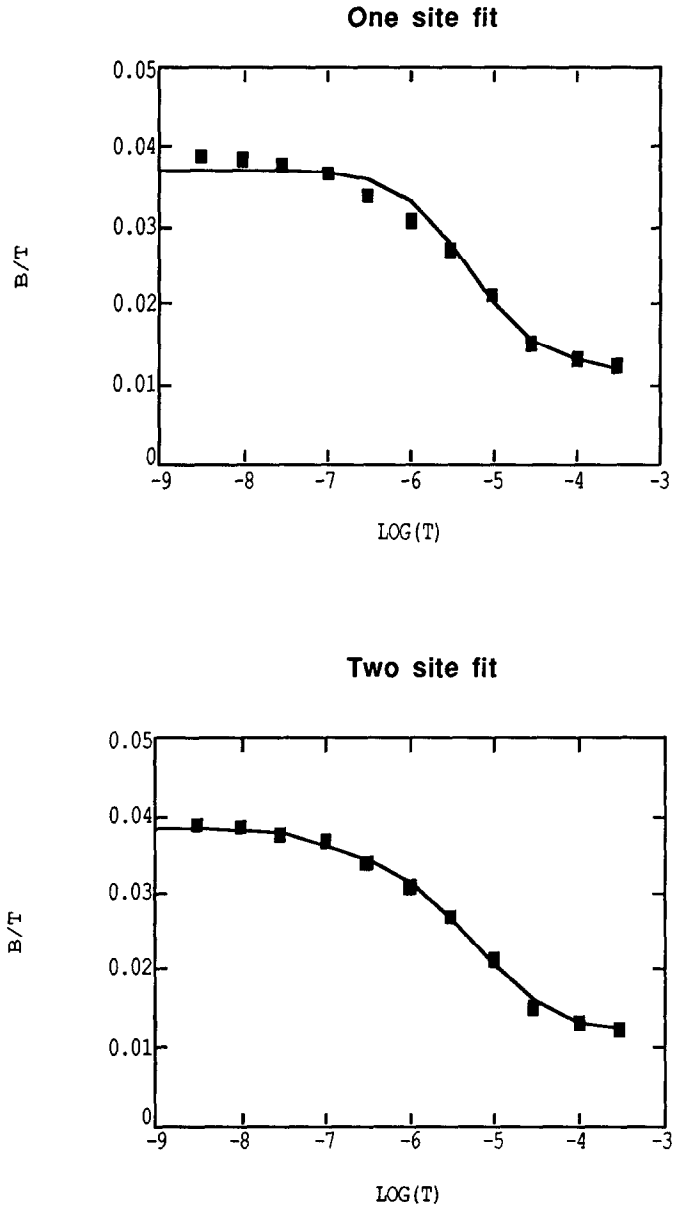


Fig. 1.
Concentration-inhibition curves from one experiment investigating ibogaine inhibition of [^3H]-naloxone binding to mouse brain membranes. Shown are one and two models of the data. The two site model provided a significantly better fit to the data than did the one site model ($p=0.002$). The high affinity component comprises about 20% of the total number of binding sites.

(14,17). Our finding of a lack of significant alteration by Na^+ of the K_d value of $[^3\text{H}]$ -naloxone is in accordance with its known antagonist function. The apparent increase in naloxone's B_{max} value by Na^+ confirms previous observations (12,13) which have been attributed to Na^+ recruitment of nascent free receptors into the low affinity receptor pool (12).

Using the "sodium shift" technique, ibogaine appears to be an agonist at opioid receptors. The K_i value for ibogaine inhibition of naloxone binding is significantly poorer in the presence of Na^+ . Additional evidence that ibogaine is an opioid agonist is provided by its biphasic inhibition of $[^3\text{H}]$ -naloxone binding (Fig. 1). Ibogaine inhibition curves in Tris buffer are best fit by a two site binding model, with the high and low affinity components presumably reflecting binding to G-protein coupled and uncoupled (respectively) states of the receptor. Biphasic rather than monophasic concentration-inhibition curves generally characterize agonist inhibition of antagonist binding to receptors in the G-protein family (18). In the presence of 100 mM NaCl, however, ibogaine inhibition curves fit one site models of the data; the weak K_i value obtained from these analyses indicates that the mu receptor is in the agonist low affinity state under these conditions (Table II).

These results suggest that ibogaine has over a thousand-fold better affinity for the mu opioid receptor than previous results indicate. These earlier studies (5), reporting virtually no inhibition of $[^3\text{H}]$ -carfentanil binding by ibogaine at a concentration of 100 μM , may be misleading because of carfentanil's extremely slow rate of dissociation from the receptor (19), leading to possible underestimation of the K_i values of competing ligands. In support of this argument, the K_i values determined for several well known opioids (e.g., naloxone, morphine and DAMGO) by inhibition of $[^3\text{H}]$ -carfentanil binding (5) are 3-10-fold weaker than those obtained using $[^3\text{H}]$ -DAMGO (20) as the radiolabeled ligand. In fact, a recent report confirmed the lack of ibogaine inhibition of $[^3\text{H}]$ -carfentanil binding, but demonstrated ibogaine inhibition of $[^3\text{H}]$ -DAMGO binding in calf brain cortex (K_i value, 11.0 μM ; 6).

The present finding of a high affinity K_i value for ibogaine inhibition of opioid binding raises the possibility that interaction with the mu receptor may play a role in ibogaine's in vivo activity. While ibogaine interaction with several receptors has been reported (5,8), the present K_i value of ~130 nM is 10- to 20-fold more potent than the affinity reported for ibogaine at the kappa opioid receptor (2 μM ; 5) or at the $[^3\text{H}]$ -WIN 35,248-labeled dopamine transporter (1.5 μM , 4; 3.5 μM , 8), and is similar to the 90 nM K_i value reported for ibogaine binding to the sigma₂ site assayed in rat liver membranes (7). Thus interactions with the mu opioid receptor may significantly contribute to ibogaine's enhancement of morphine-induced antinociceptive effects as well as its reported reduction of opioid withdrawal symptoms and attenuation of drug seeking behavior.

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